Before you start & General information

If you have not received MALDI training, email Jim Windeck

There is a good, easy-to-use guide for inserting the MALDI plate and basics on the MALDI home page

The MALDI room can be opened with you NMR key if it is locked

McNeil lab materials

MALDI plate: 2615 wooden shelves

DCTB (matrix) 2615 fridge (prepare your own stock solution - see below)

Pipetter (pink, 2.5 µL): one in 2623, one in 2615

Pipette tips: above the DSC (take your own box)

Eppendorf tubes: 2615 wooden shelves

*please return supplies to their locations after using (exceptions - pipette tips & DCTB Stock Solution)

Cleaning the MALDI plate

If the MALDI plate needs cleaning, use a solvent that dissolves the polymers and does not tarnish the plate (i.e., THF). $CHCl_3$ works well. Scrub the plate (gently) with a chemwipes and Q-tips with at least 3 washes. Sonicating can also help. Let the plate air dry outside of your hood. If you see any remaining polymer on the plate continue washing until no polymer remains. Note, the plate does take time to dry so please give yourself & others time (otherwise samples run).

DCTB stock solution

Most polymer samples will ionize well using the matrix *trans*-2-[3-(4-*tert*-butylphenyl)-2-methyl-2-propenylidene]malononitrile (DCTB). Weigh 5–10 mg DCTB into a 4 or 8 mL vial and dissolve in a minimal amount of $CHCl_3$ (add dropwise). Store your stock solution in the fridge. If the amount of DCTB remaining is low after you make your stock solution **order more immediately**! DCTB is often backordered and is essential for MALDI. Anthracene can also be used as a matrix; however, McNeil members have had better results with DCTB.

Sample preparation

Your sample should be completely dissolved and separated from salts - salts (e.g., Mg, Na) can ionize with your sample, so to simplify analysis filter your samples before preparing the MALDI plate. Note: filtering does not ensure salts will *not* be present but can help minimize their occurance. For most polymers $CHCl_3$, or the GPC prep (THF:PhMe (99:1 v/v)) works well to dissolve polymer samples. Prepare the MALDI plate by mixing dissolved polymer (~1–3 mg polymer/1 mL THF, 2.5–10 µL) with DCTB dissolved in $CHCl_3$ (2.5–5 µL). Use the 2.5 µm pipette to mix samples with matrix. Diluted samples with varying polymer/DCTB ratios (to ensure good signal/noise), spot on the MALDI 96-well plate, air dry.

Calibrating the Instrument:

Can be done before data acquisition (calibrates the instrument) OR during data work up (calibrates the data you have acquired). Both methods require acquiring a spectrum from a standard. Note: you should calibrate for all of your data, specifically for RP vs LP (laser power can affect peak placement), and for the specific instrument session. Calibrating usually does not significantly shift spectra i.e., typically <3 Da. If you see a drastic change (>8 Da) double check your work, if it persists consult a friend/Jim. It is recommended to make/use a standard polythiophene (Br/H) or phenylene (Br/H) polymer -- Former McNeil member (Peter Goldberg) made calibration curves for P3HT Br/H, P3DT Br/H, polyphenylene (PPP) Br/H and these polymers can be ionized using the same methods you will likely use for analysis.

**You can also make your own calibration curve.

**Calibrating the instrument before data acquisition.

Calibrating during data work up (see data work-up section)

Calibrating notes:

- If you see isotopic resolution - each mass peak has many peaks representing isotopes, typically data from RP (not LP) methods - use SNAP processing (determines mass at the *start* of the mass peak). If your masses have a single point (i.e., all isotopes blend together - typically LP, also RP if have poorer resolution) use centroid (determines mass at the *center* of the mass peak).
- There are different calibration curves for SNAP versus centroid peak picking.

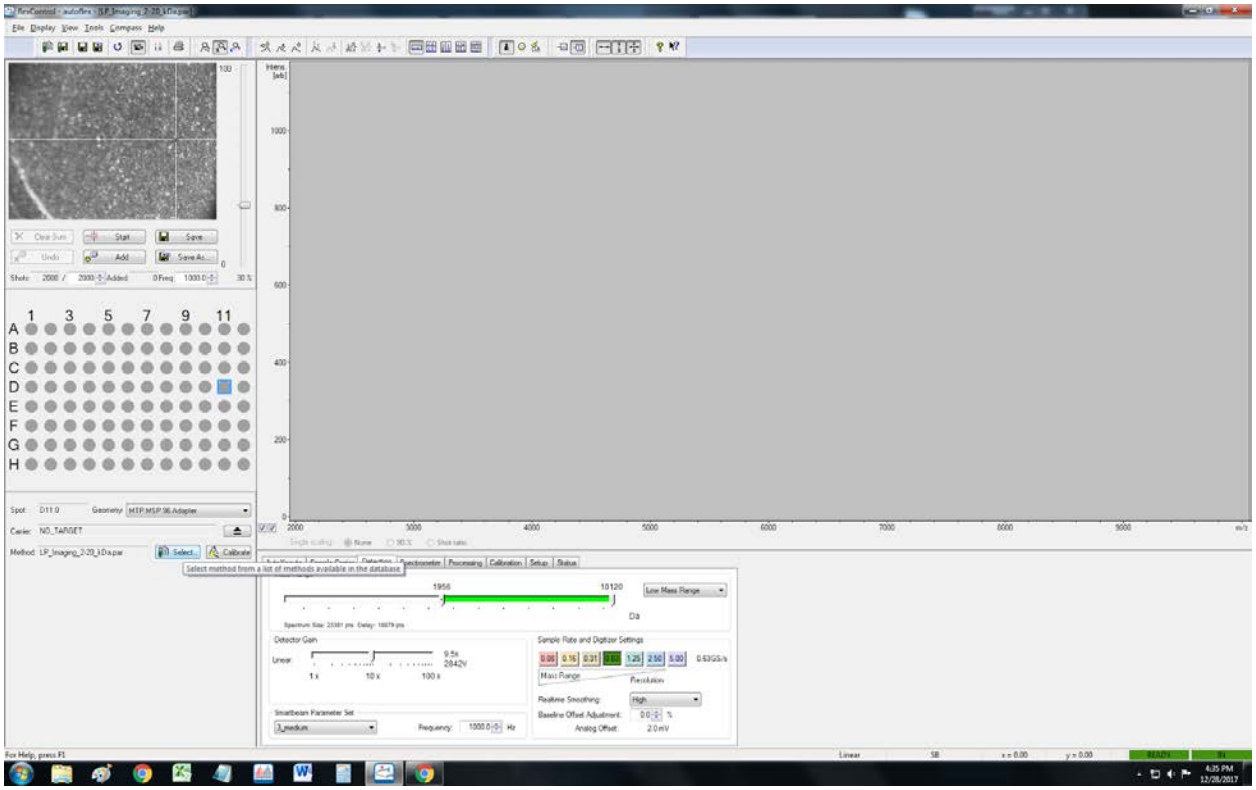
- When working up your data make sure to calculate theoretical masses based on the correct values (SNAP or centroid). From ChemDraw, these values can be calculated using exact mass (SNAP) or mol. wt. (centroid) in the analysis window.

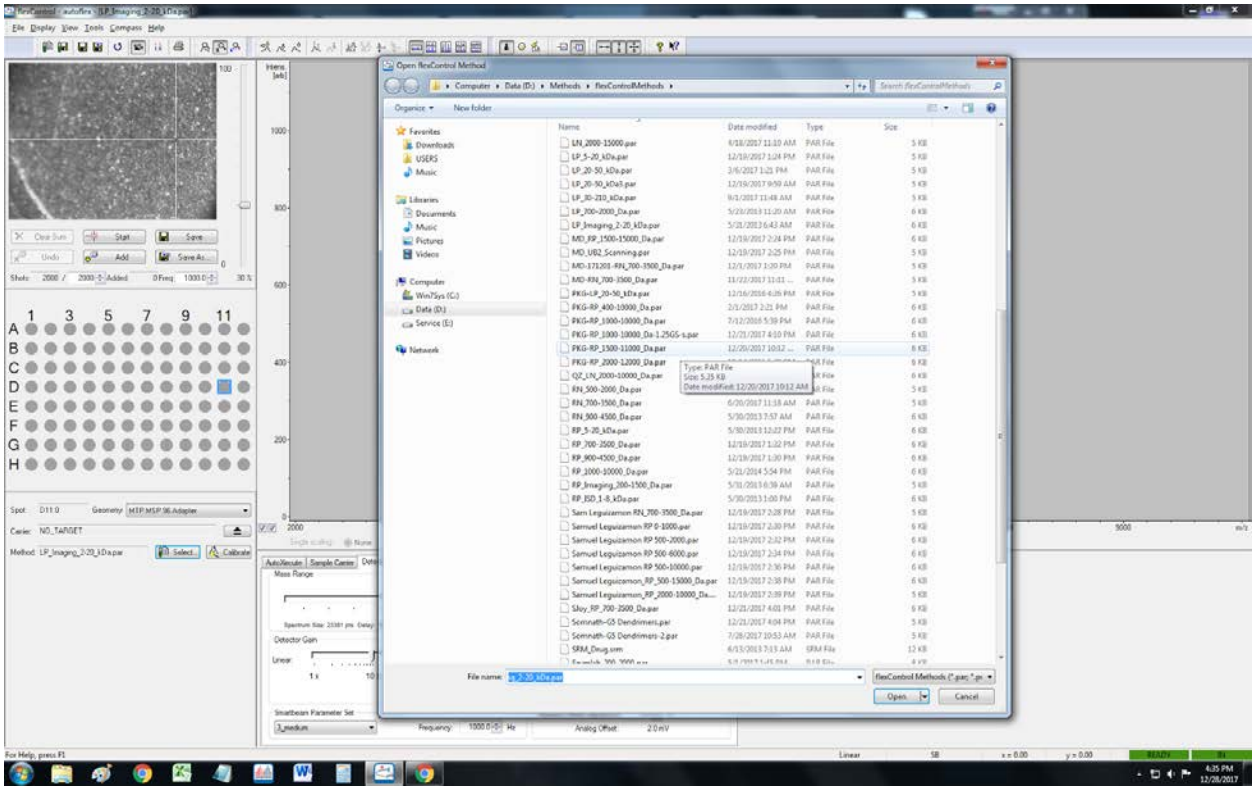
Using the MALDI

After your plate is in the instrument, and the vacuum is below 2.0 X 10^-6 torr, you can begin.

Step 1: Choose a method

Click [select] to select your method. Methods vary on laser power - regardless of your method choice start with a lower laser setting (see laser power Step 5) and work up to higher strengths as necessary.





RP - innately lower laser power:

Polymers >1000 Da: Former McNeil member (Peter Goldberg) made RP methods (PKG RP….) these have good laser settings to ionize polymers within the ~defined MW ranges. They sample the whole plate (see step 3).

<1000 Da: try RPimg - samples a single point (see step 3)

LP - innately higher laser power:

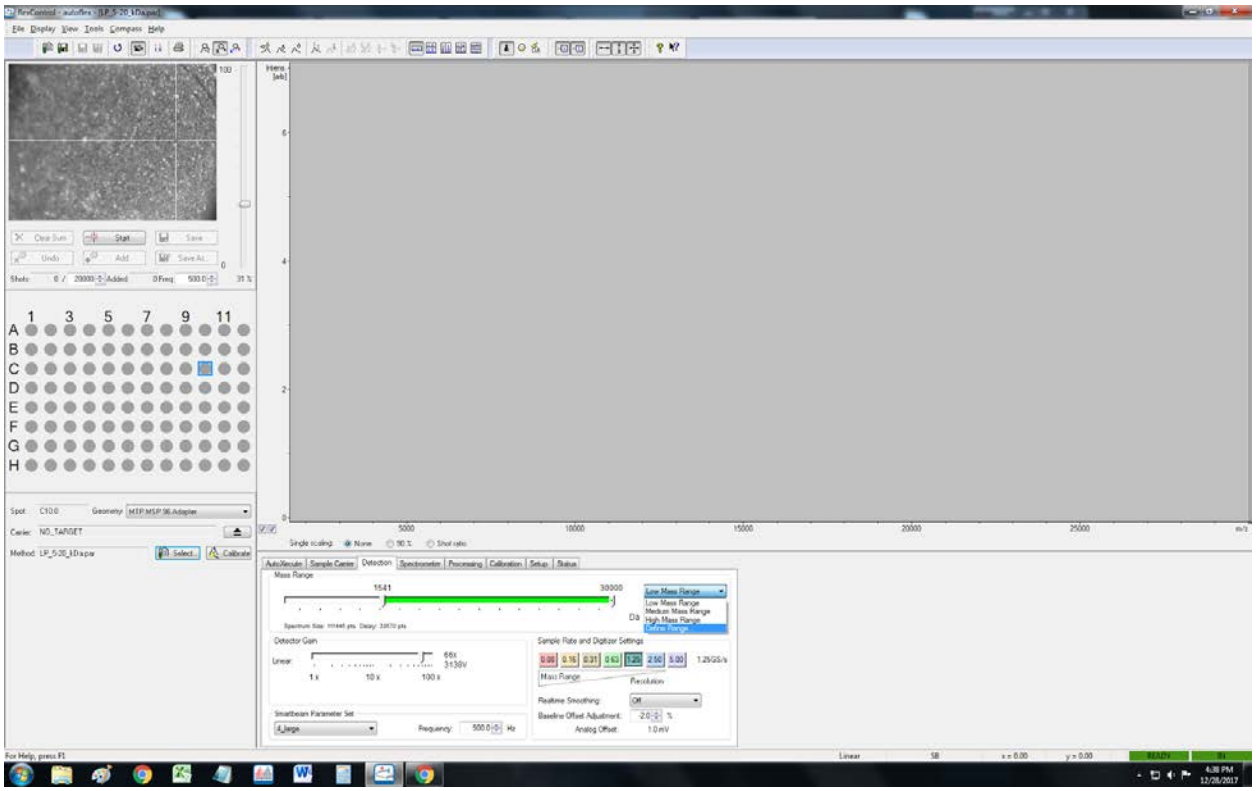
If your having a hard time getting a good signal-to-noise try LP methods rather than RP methods.

Polymers >1000 Da: There are various LP methods/LPimg have defined mass Ranges.

<1000 Da: try LP700-2000

Step 2: define mass range

This can be done using the mass range scale (detection tab).



Often there is low molecular weight *noise*, likely some related to the matrix, it can help to define a “limit” for the mass range observed. This can be found in the spectrometer tab.

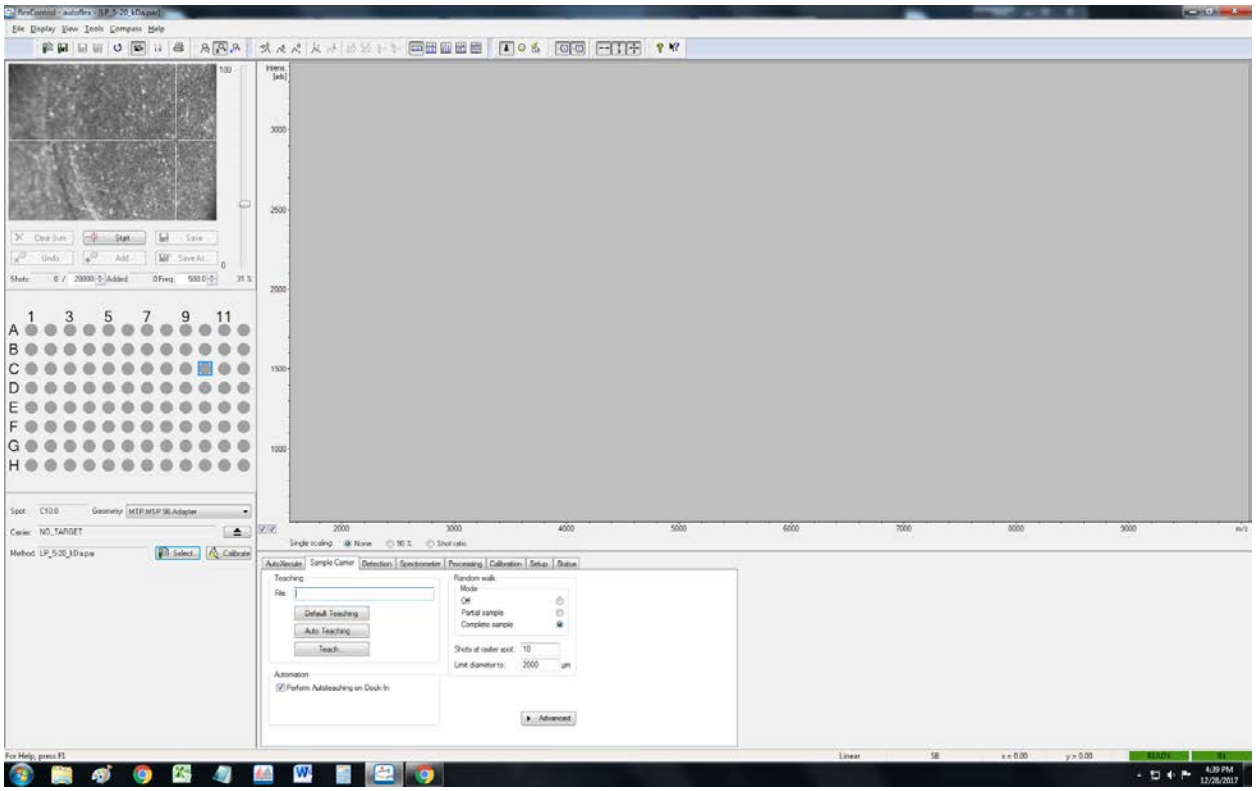
Notes:

- Selecting a narrower window (cutting out high MW where you have no sample) should not affect the signal-to-noise of your sample (as it would with NMR).
- Changing your window will delete your “summed spectra” so make sure to save your sum before changing your mass range.

Step 3: whole plate/ single spot

Sample carrier tab. Define having the laser randomly sample places while shooting (complete sample - innately lower power) versus shooting laser in a single spot (partial sample - innately higher power) on the plate. In either case, after the sequence is complete, click on a different point on the spot (in the camera view top left corner crosshair indicates from where laser samples) then run the sequence again. [Add] the sequences together to give a *realistic* view of

what is on your plate, and will reduce signal to noise. If you do not click in a different place, you will be shooting in a place where the laser has already deteriorated/worn through the sample.



Step 4: number of “shots”/ frequency

Adjust these values in response to your signal-to-noise. Typically good values to start with are 1500 “shots” and 500 “freq.” These are found in the top left hand corner of your screen under the visual of the MALDI plate. Note: many LP methods are automatically set to 20000 shots, you can hit [Stop] during the middle of a run if your sample is deteriorating, and your data is saveable. Typically increasing the number of scans will improve the signal-to-noise. If you did not plate a an abundance of sample on your plate however, too many scans can wear through your sample and then decrease signal-to-noise b/c the laser hits the plate.

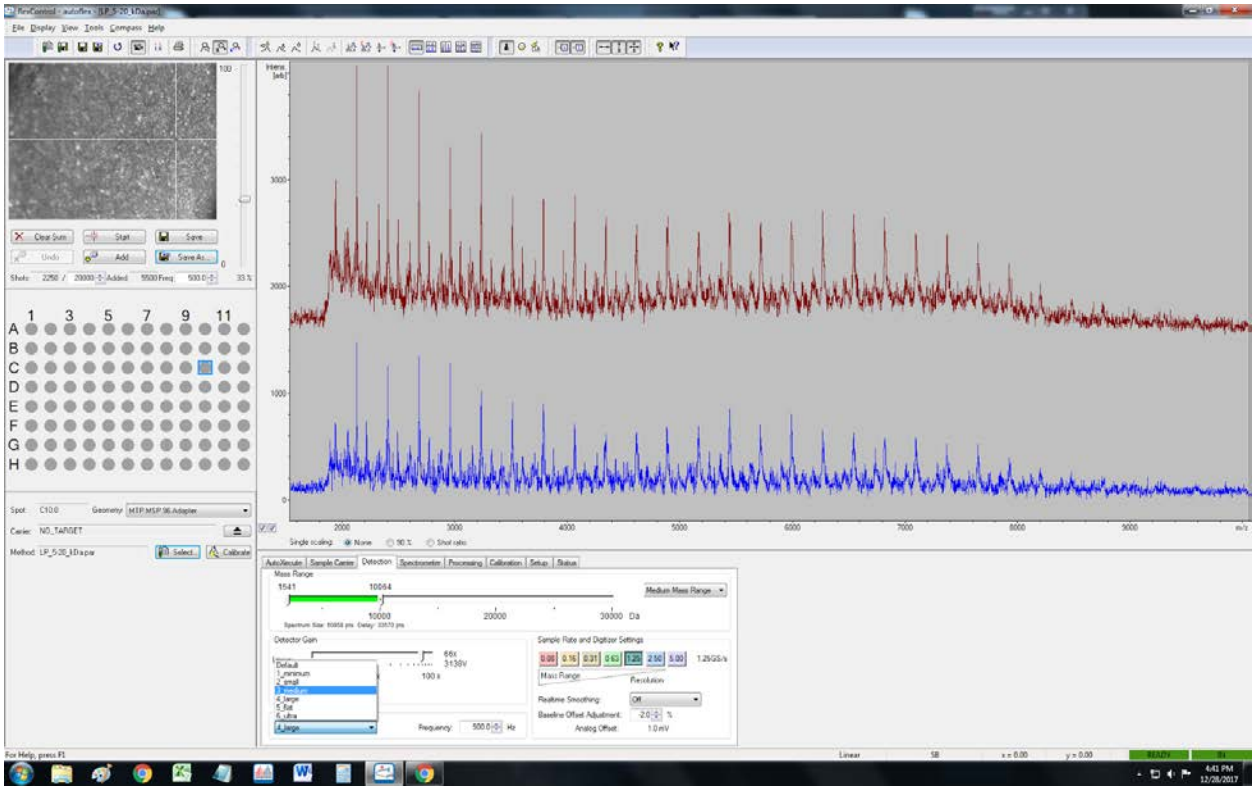
You can [Add] scans together - recommended - to reduce signal to noise. The Added slot will tell you the sum of your “shots.” When saving your data, you can specify single or sum (top left in the save pop-up) to save the most recent set of shots or the sum of all added shots respectively.

Step 5: laser power

Use the laser power bar (top left corner next to the view of your plate) to “fine-tune” the laser strength. As mentioned above, start low (RP ~20%, LP ~6%) then increase as necessary. If you see no peaks at all on your first try increasing strength by ~10–20%. If there are no peaks at high power (RP ~85%, LP~35%) either try selecting a different method or change your # of beams (see Step 6). If all else fails, a different ratio of DCTB/polymer is your best bet.

Step 6: 4 large, 3 medium

These are different beam settings (detection tab). If you are having a hard time ionizing a sample, try toggling to a different beam setting. It is unclear why one might work better than the other, please let others know if you figure it out. 3-medium appears to be higher power than 4-large. Unclear if our MALDI is equipt to do 5-XX…(these spectra are examples of bad data! See below for explanations)



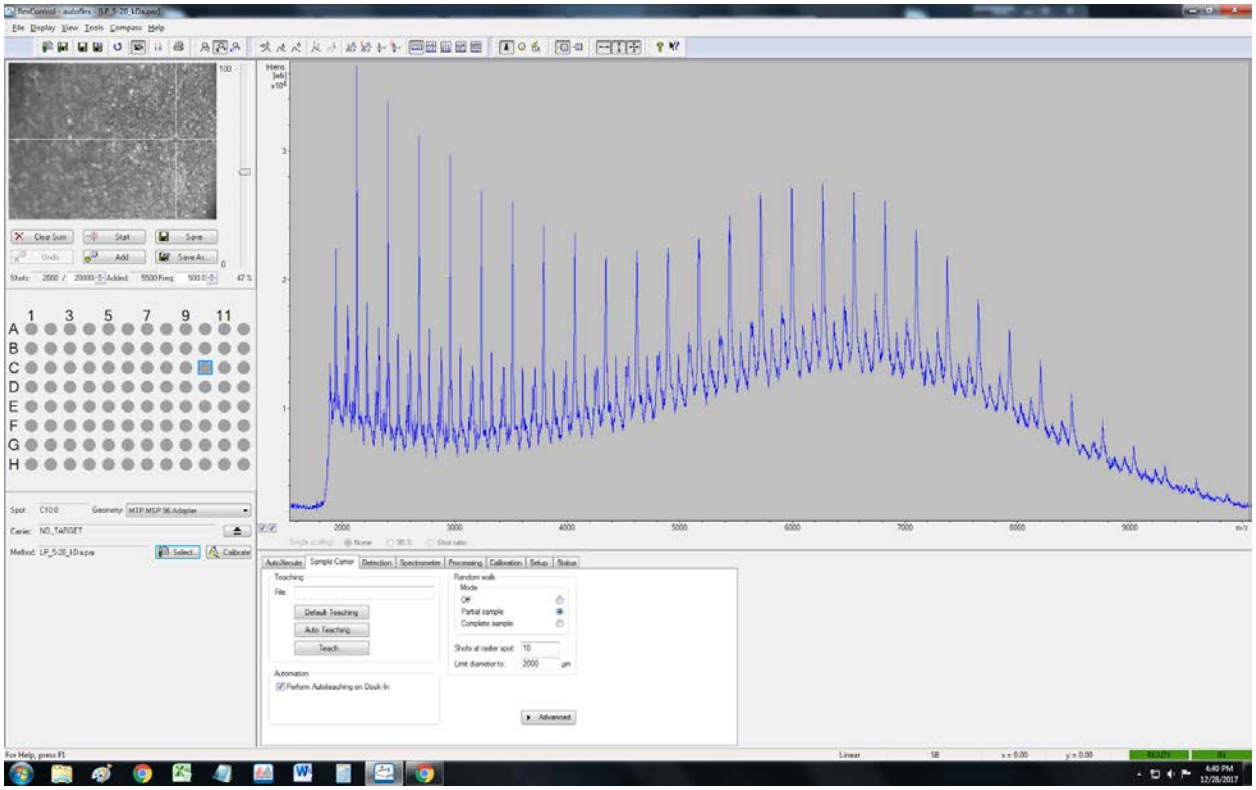
Step 7: Suggestions on saving your data

[Save] in the top left corner. Use your own folder, save *all* of your data in this folder. Make a subfolder for each of your notebooks. Make subfolders for notebook pages/ experiment types/ catalysts/ polymers -- whatever will be easiest for future data finding! Save your data with its **notebook page name**, and **method used to acquire** the data. The method can explain why you see the data you are seeing. It might be helpful to save how the sample was prepared (e.g., solvent/matrix ratio).

What to look for in your data

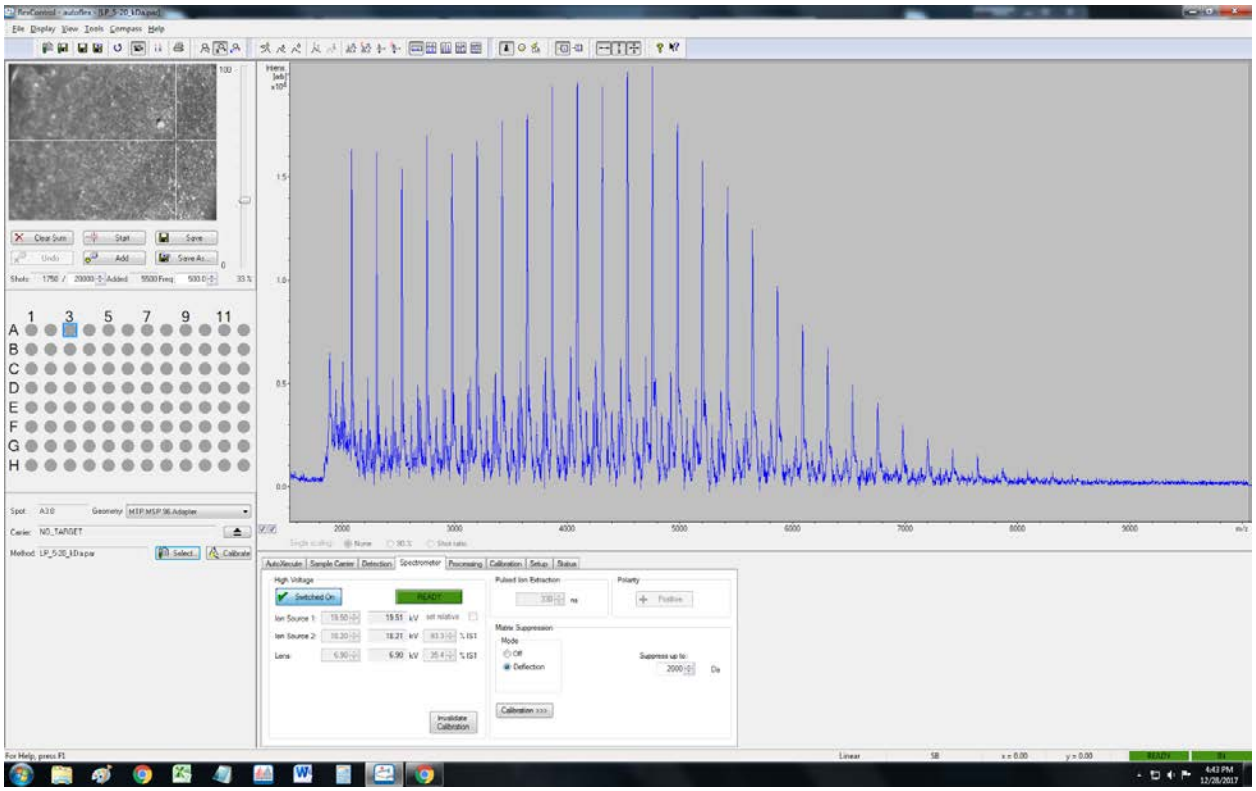
Baseline:

- Is your baseline flat? - *good!*
- Is your baseline fuzzy? - *add more scans and/or increase laser power to improve your signal-to-noise*
- Does your baseline look sigmoidal/ super squiggly? - *bad! - lower laser power/change method. Baselines can be zeroed, however a really messy baseline can often be fixed by changing the laser power.*

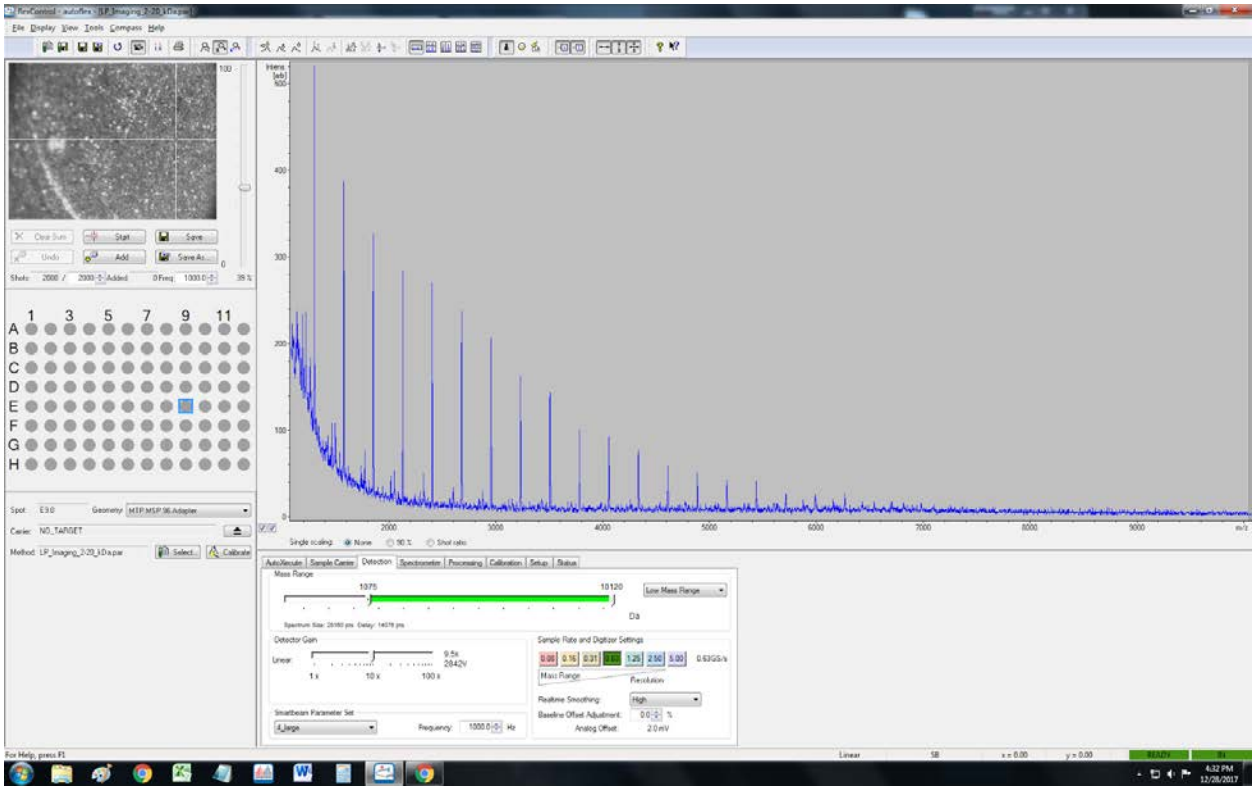


Peak shape:

- Matches your GPC trace? - *good!*
- Peak saturation (i.e., the top of the peak is maxing out in intensity)? - *lower laser power*
- Is a blob? Bad - *likely too high of laser power especially if your baseline is also wonky (this example is only a moderate blob)*



- A slanted line low MW (highest intensity) decreasing to low MW - *try changing laser powers/methods, but you likely need more matrix relative to polymer.*

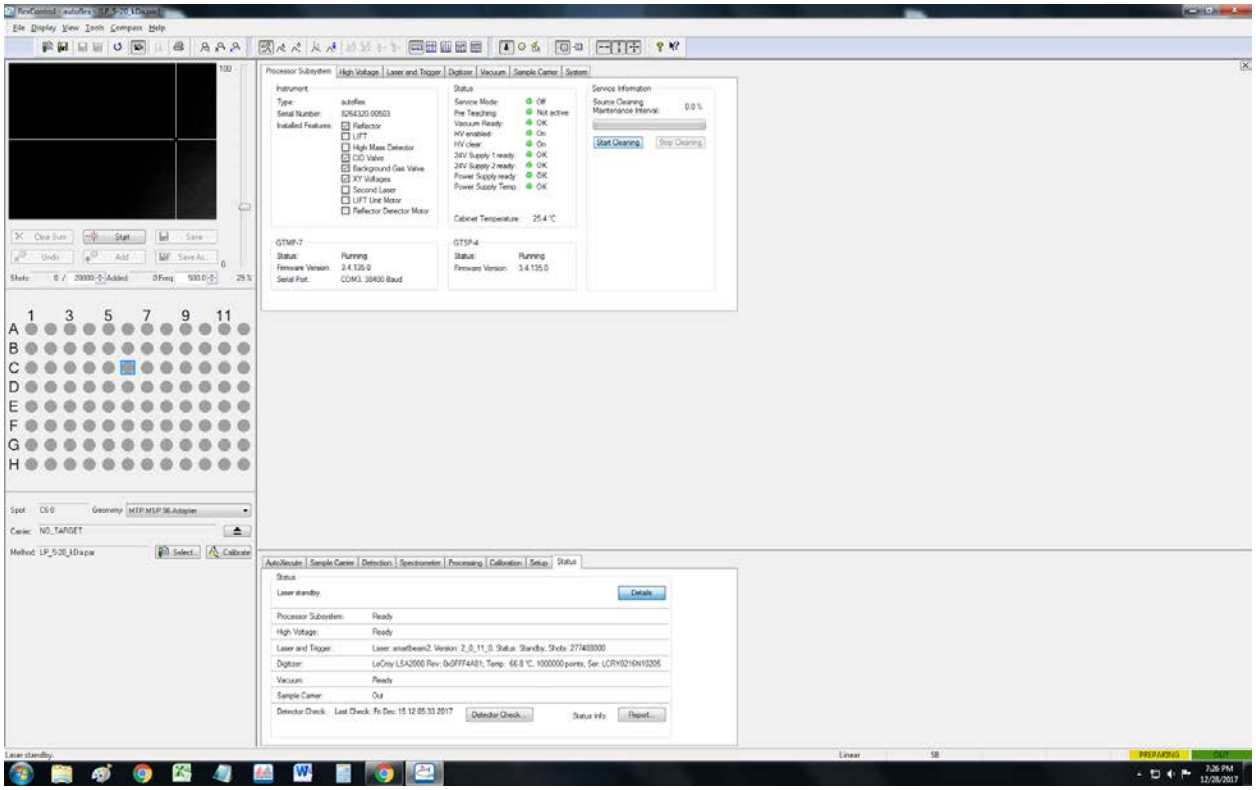


MW make ~sense:

If you shoot laser at the same spot too many times, or too high of laser power you can see peaks that do not make sense from deteriorating your sample. Try shooting at lower laser power in a fresh place on the spot. If these peaks appear at lower laser powers and fresh samples as well they are probably real.

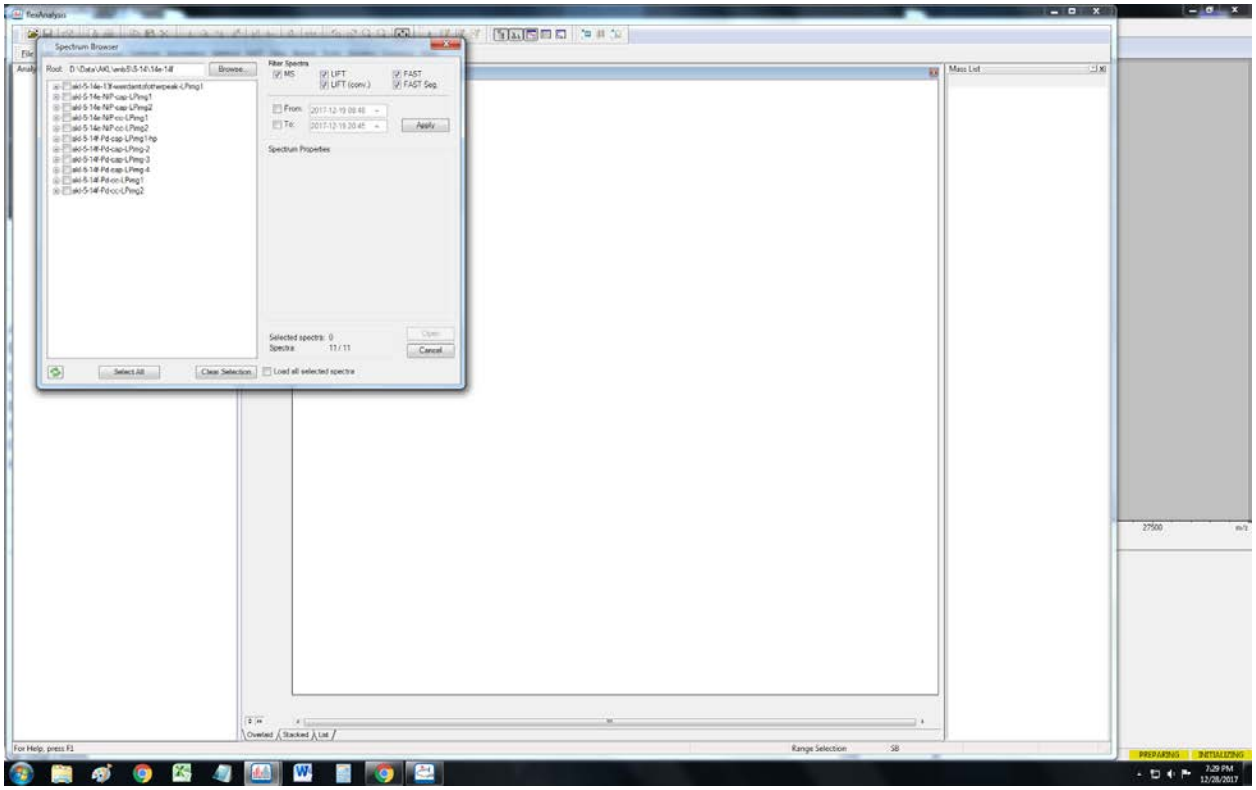
MALDI maintenance:

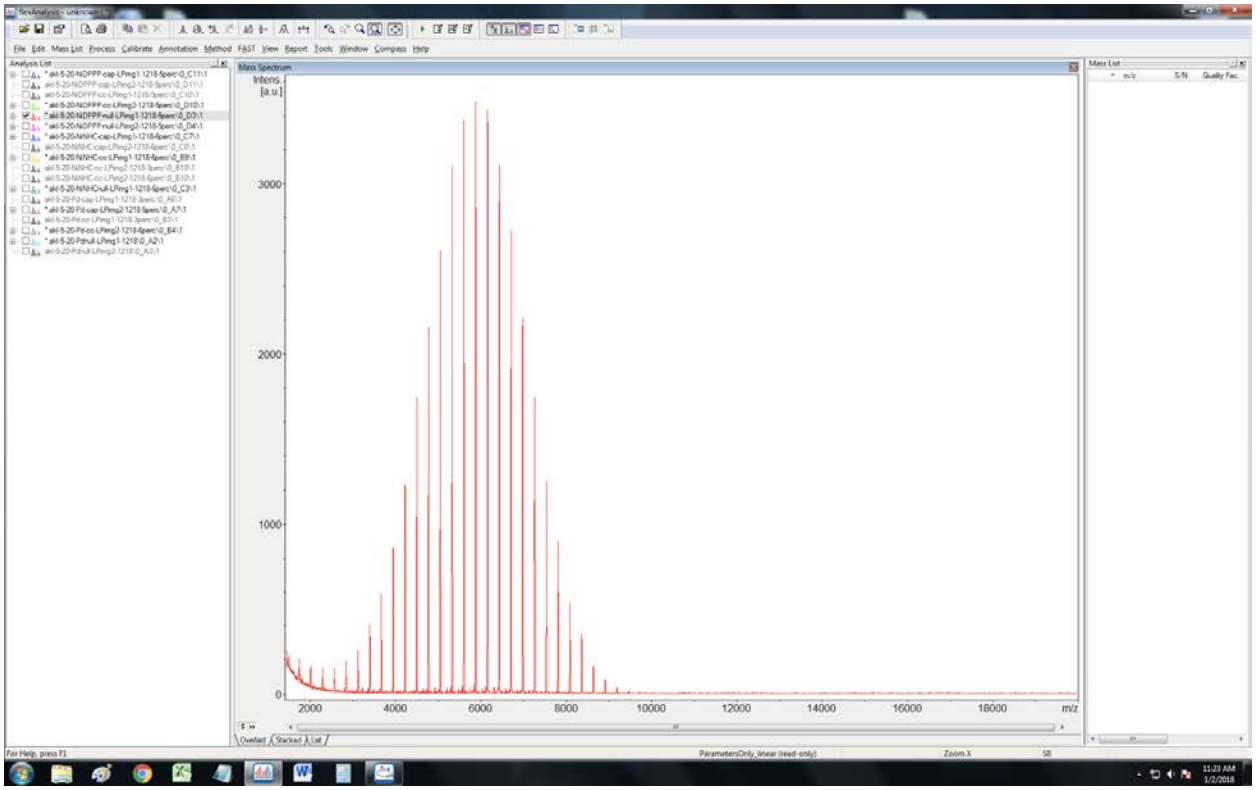
Source cleaning - you may notice a “clean your source” message. Please give yourself 20 min to do the source cleaning, or tell Jim. Cleaning can be done by choosing the status tab (far right)-> click details (top right button). This window will appear. If the source cleaning maintenance interval is >80% (top right in this pic at 0.0%) press clean source (button under the status bar).



Data Work-up Part 1: Flex analysis

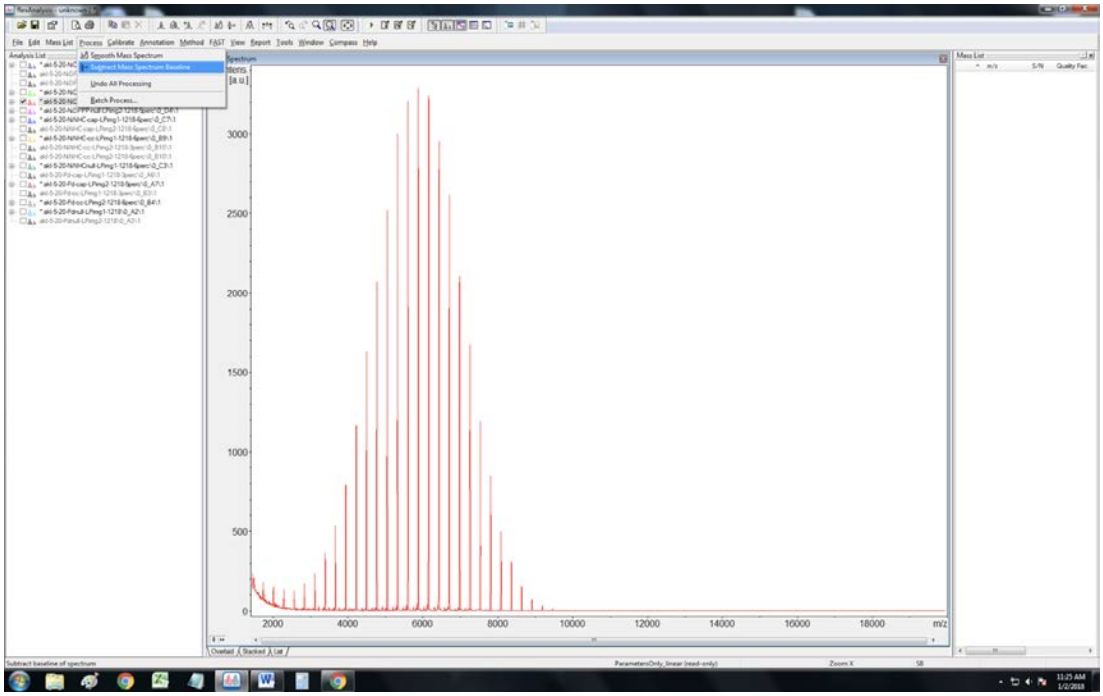
1. Open your data
    a. If you cannot find your data double check the “date acquired” boxes are either unchecked or are set for when your data was acquired (the ‘From’ and ‘To’ boxes, click [Apply])

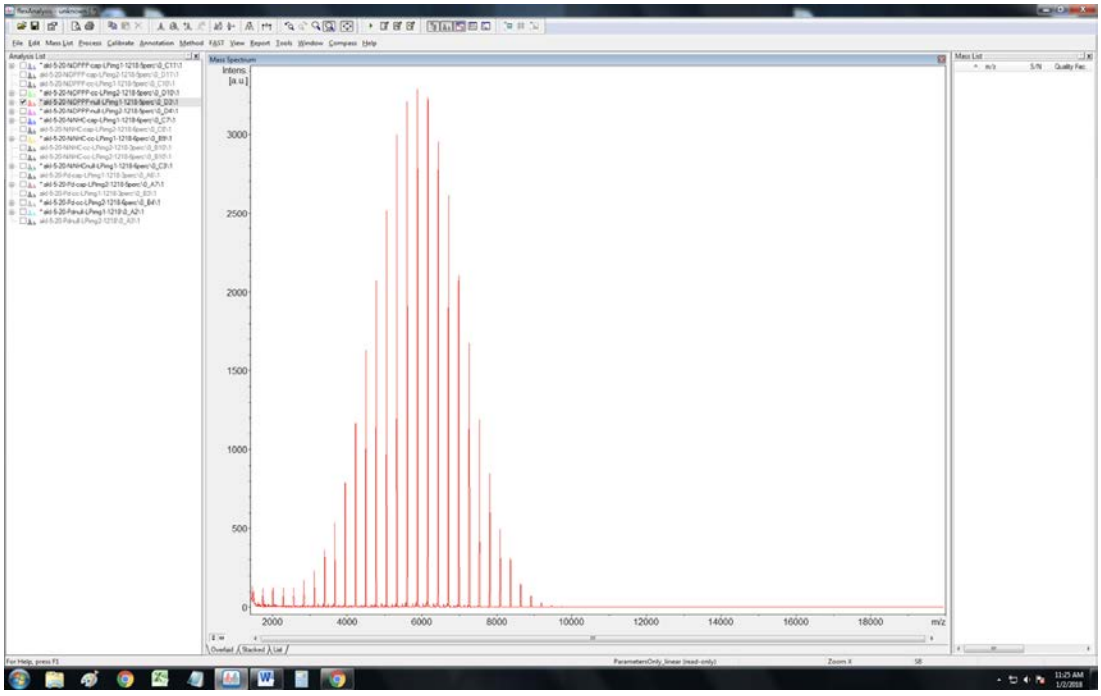




2. Process
    a. Smooth the data (smooth mass spectrum)

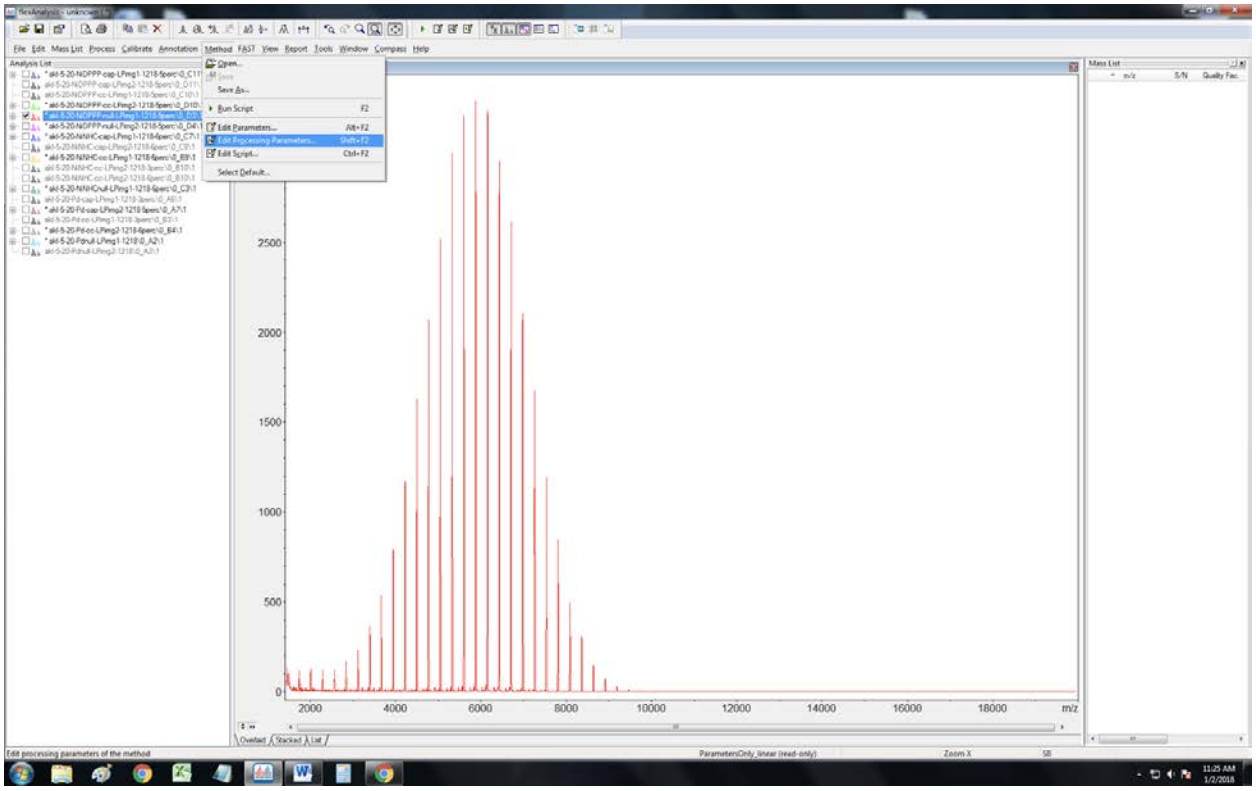
b. Zero the baseline (subtract mass spectrum baseline)





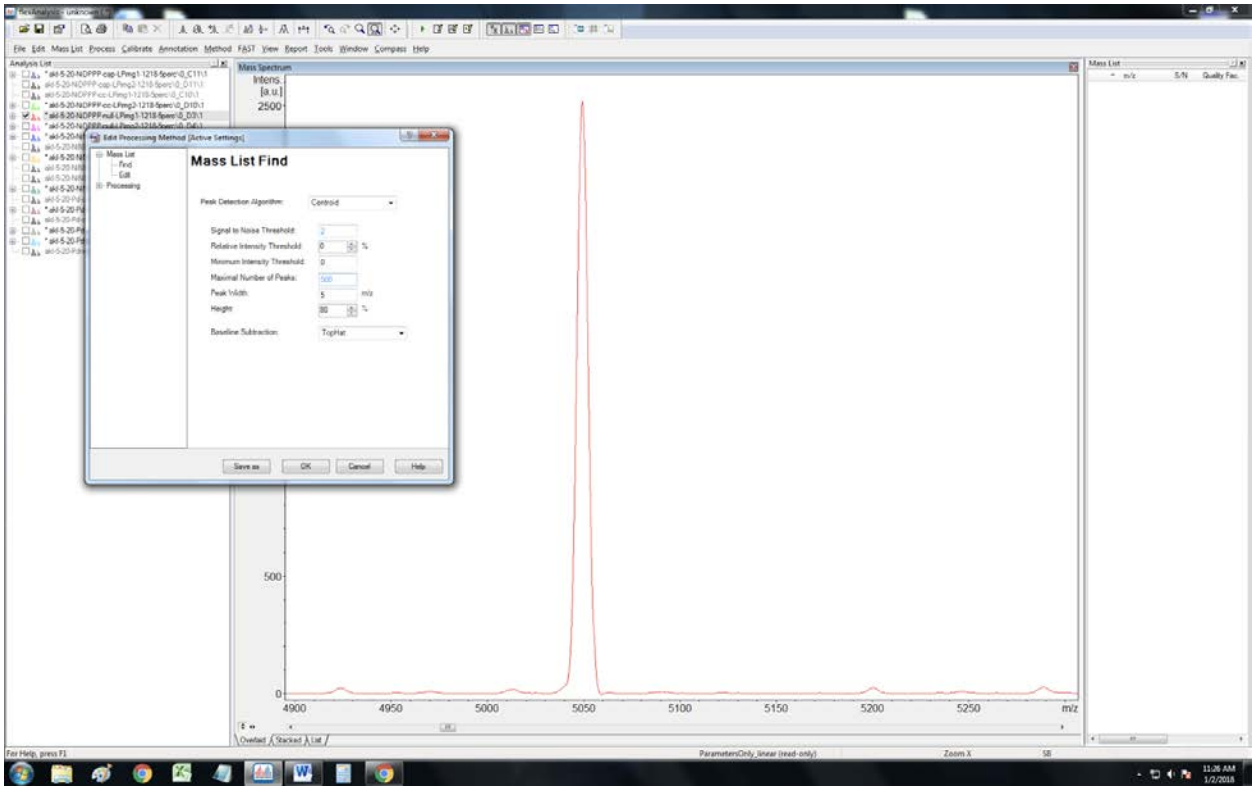
(data is processed)

3. Set find mass parameters (method -> edit processing parameters)

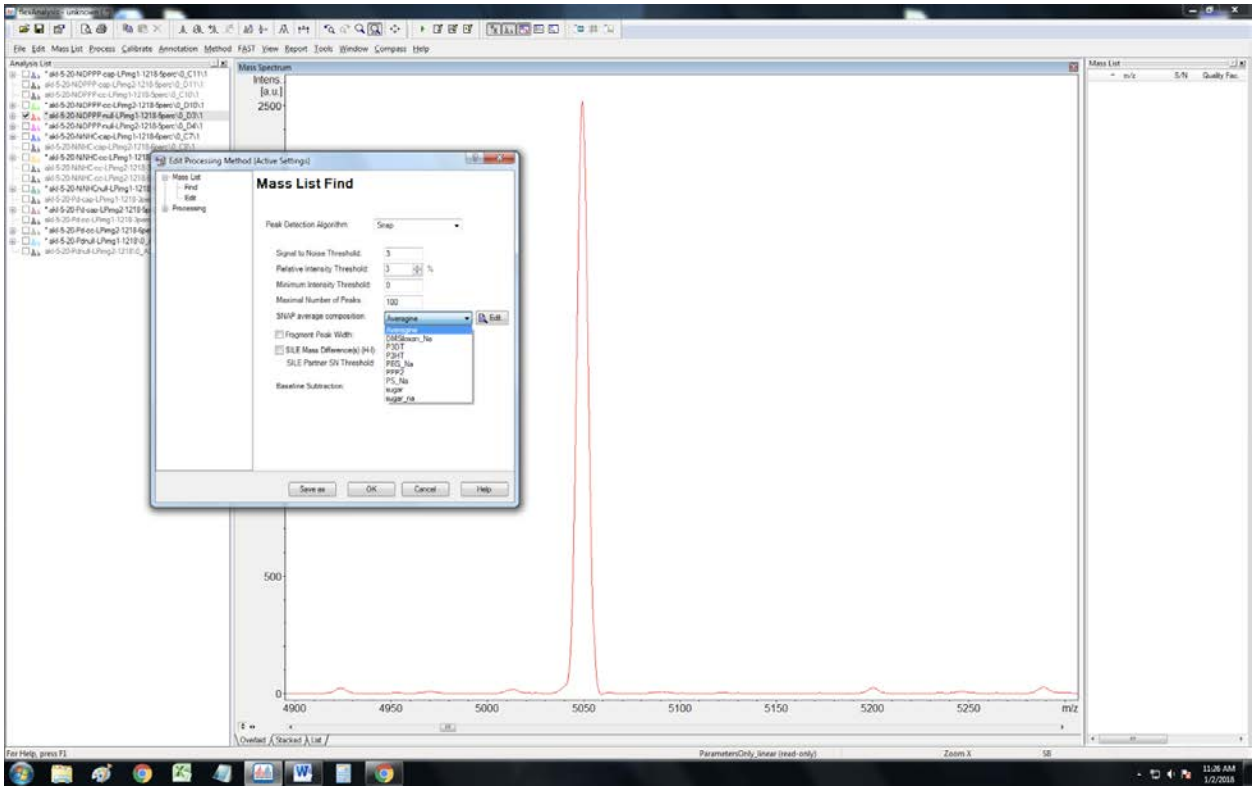


a. Define SNAP or Centroid

i. Recall: If you see isotopic resolution (each mass peak has many peaks representing isotopes) (typically data from RP (not LP) methods) use SNAP processing (determines mass at the *start* of the mass peak). If your masses have a single point (i.e., all isotopes blend together - typically LP, also RP if have poorer resolution) use centroid (determines mass at the *center* of the mass peak). Note there are different calibrations for these. Also when working up your data make sure to calculate based on either the SNAP or centroid value. From ChemDraw, these values can be calculated using exact mass (SNAP) or mol. wt. (centroid). (in the img below, there is no isotopic resolution on peak - i.e., centroid)



ii. SNAP - pick your sample type (in the dropdown) (no isotopic resolution so not using snap, but here is the window you would see)

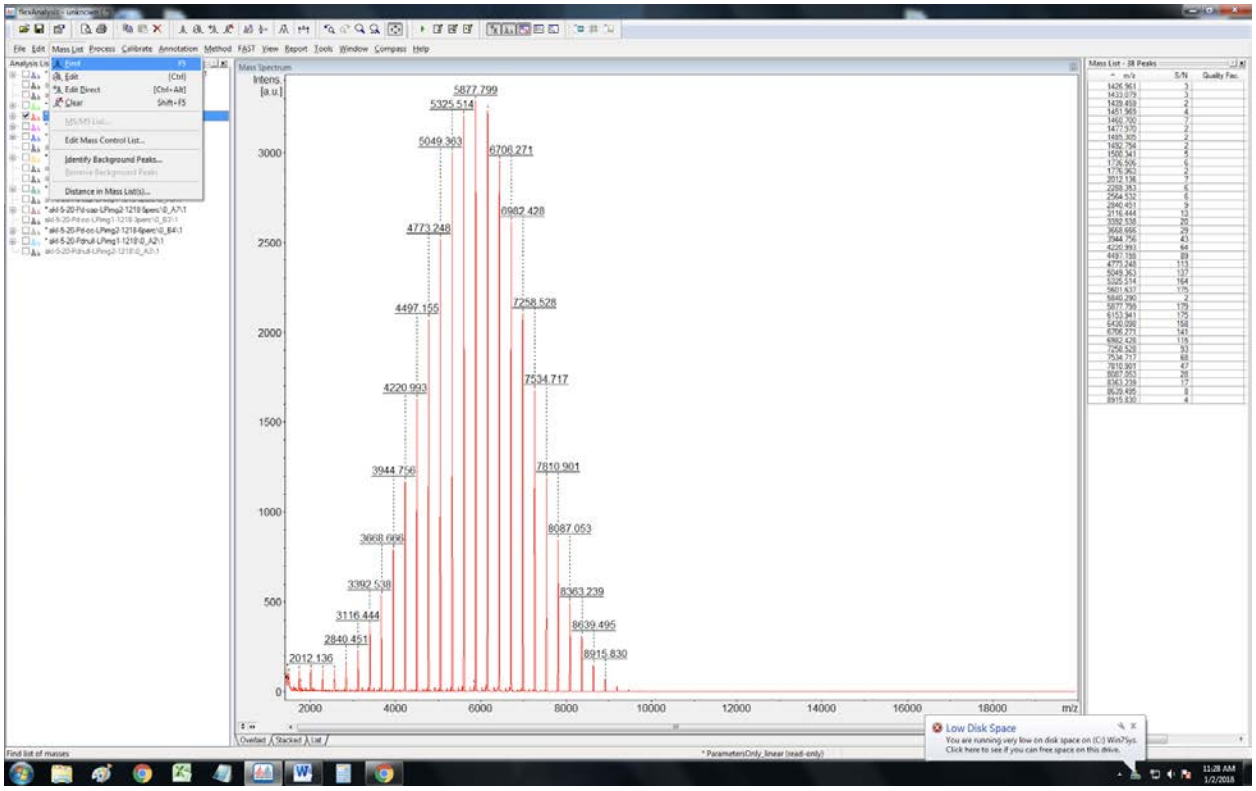


b. Signal-to-noise for peak picking - typically pick either 1 or 2

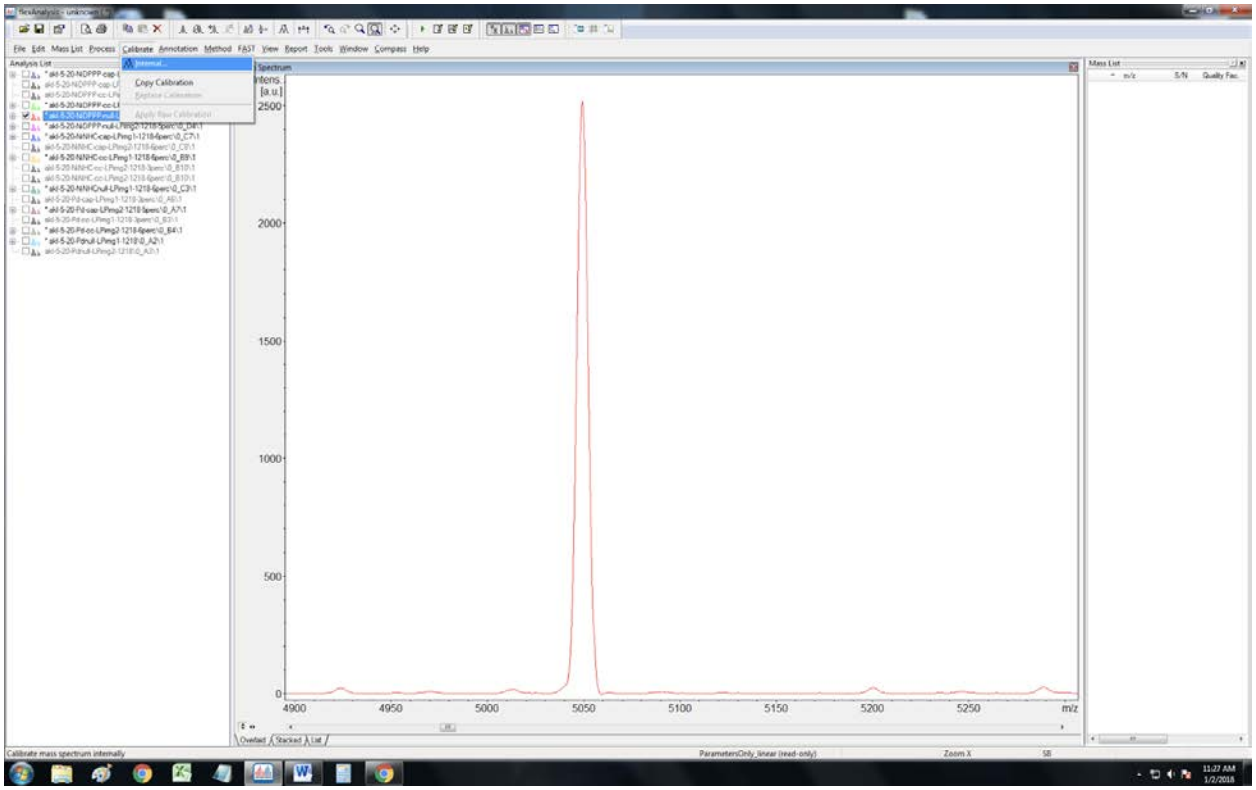
c. Number of peaks - automatically is set at 100, if your data is a simple, low dispersity curve this will be enough, but it does not hurt to increase the value to ~500 to ensure accounting for the full curve.

4. Calibrate (using your standard)

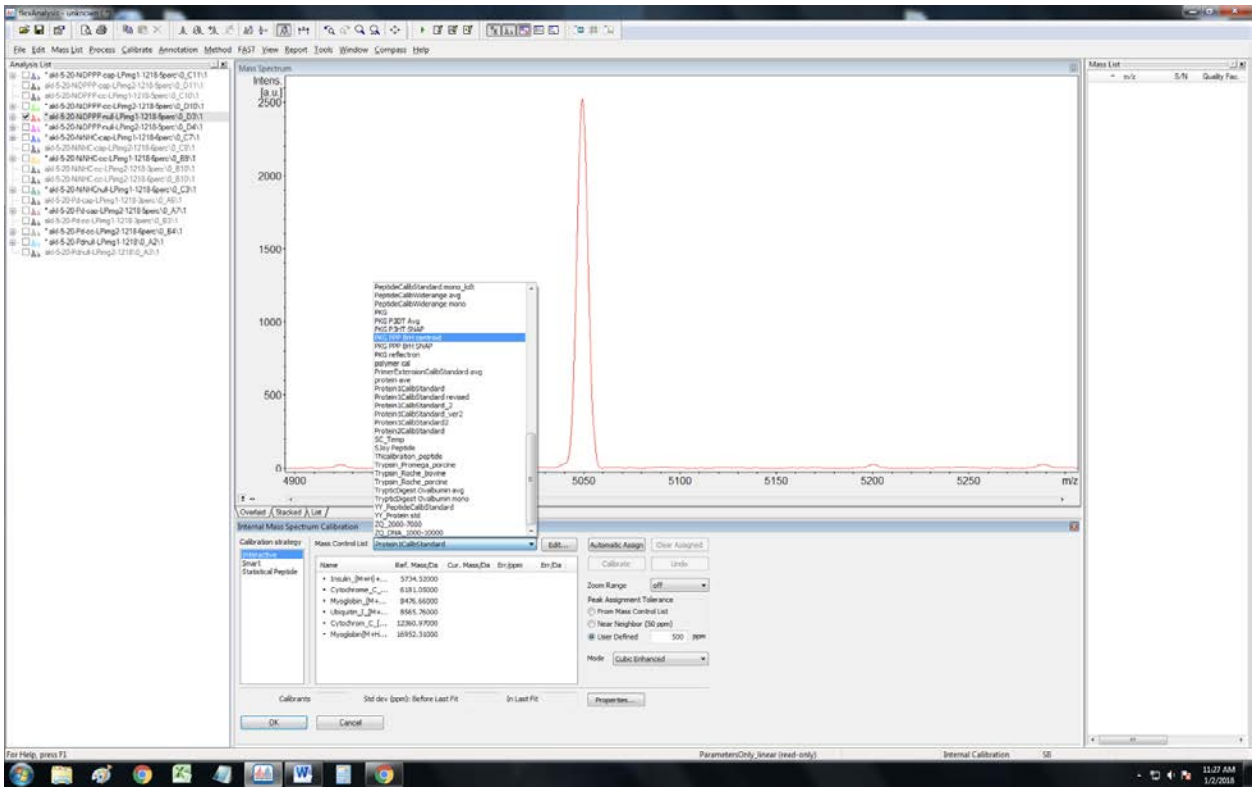
a. find masses (F5)



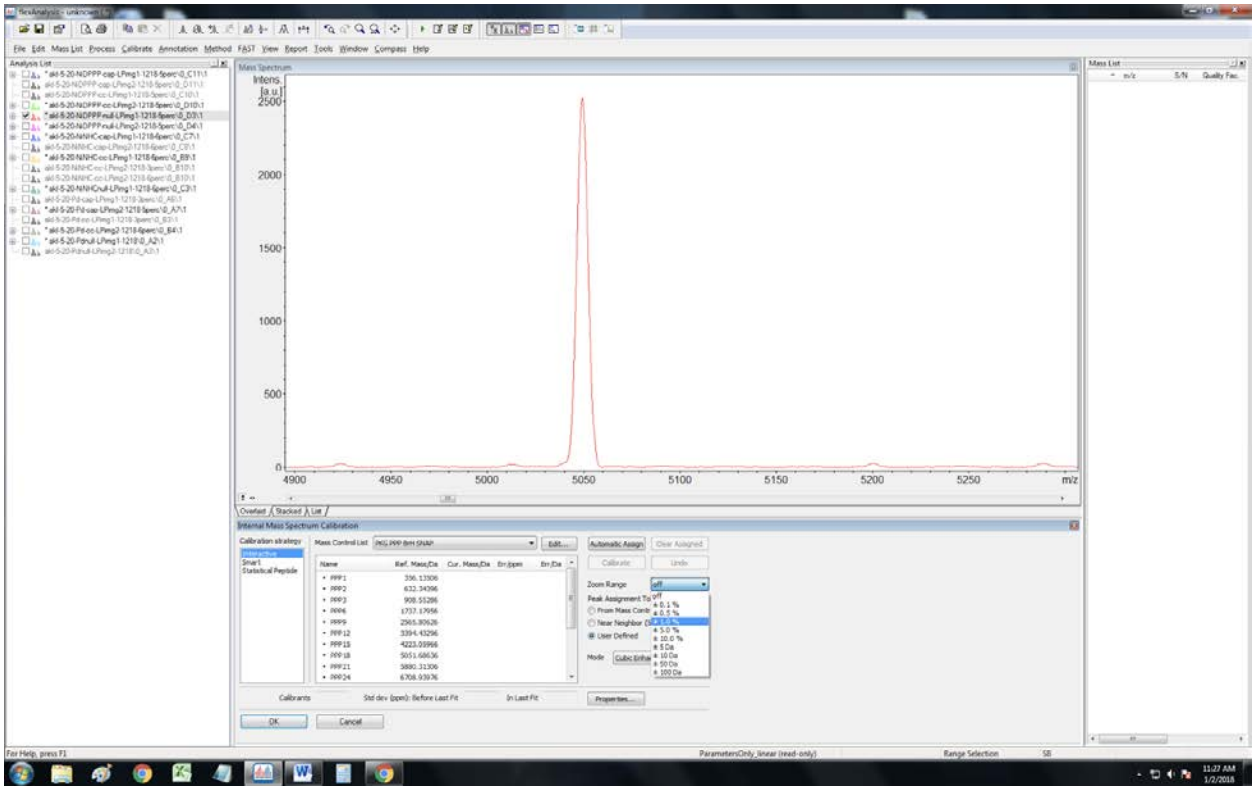
b. Calibrate -> internal calibration



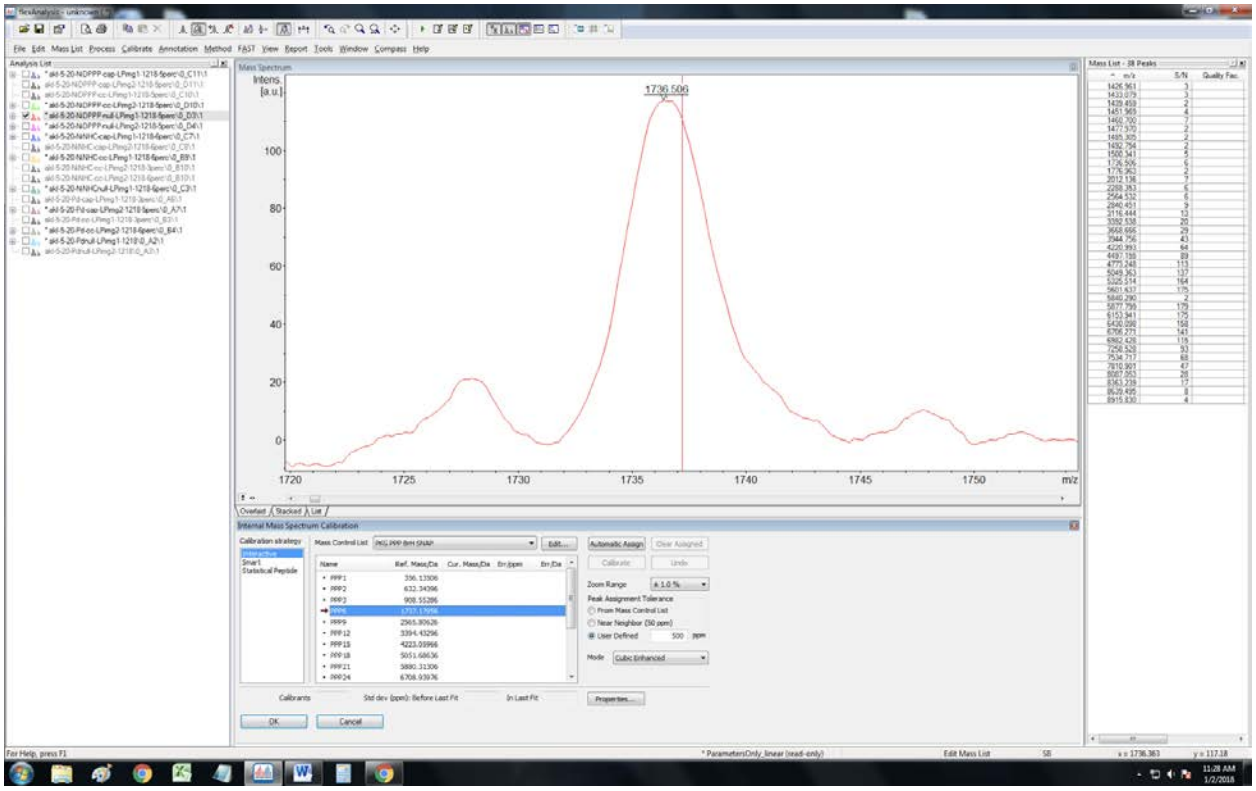
c. Choose your sample type



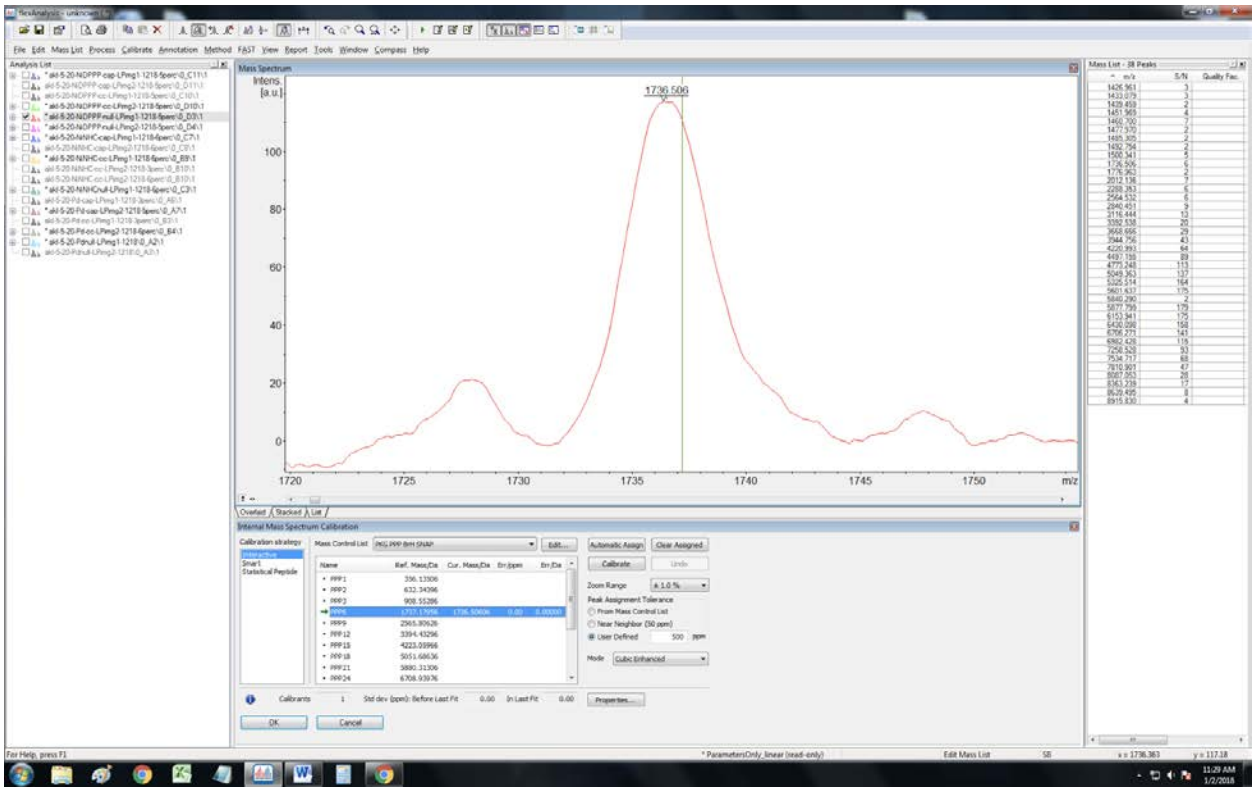
d. Define the range the server will show you (typically w/in 1.0% is sufficient)



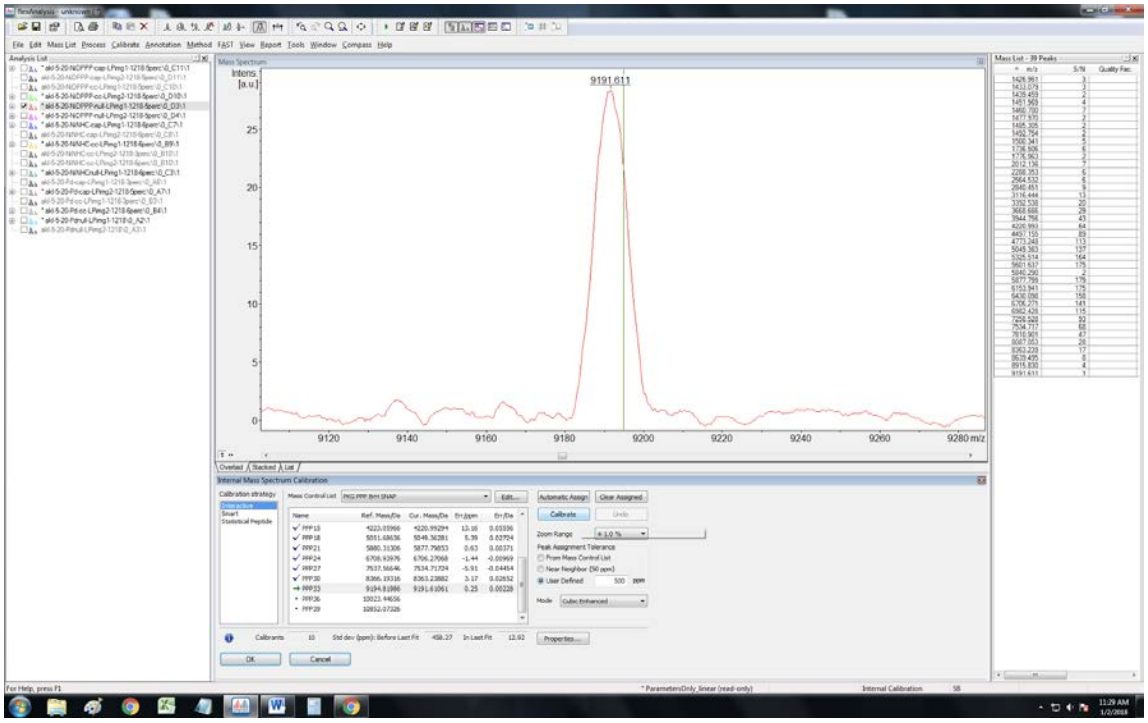
e. Click on the mass in the list, a redline will appear where that mass is and your window will show the closest peak.

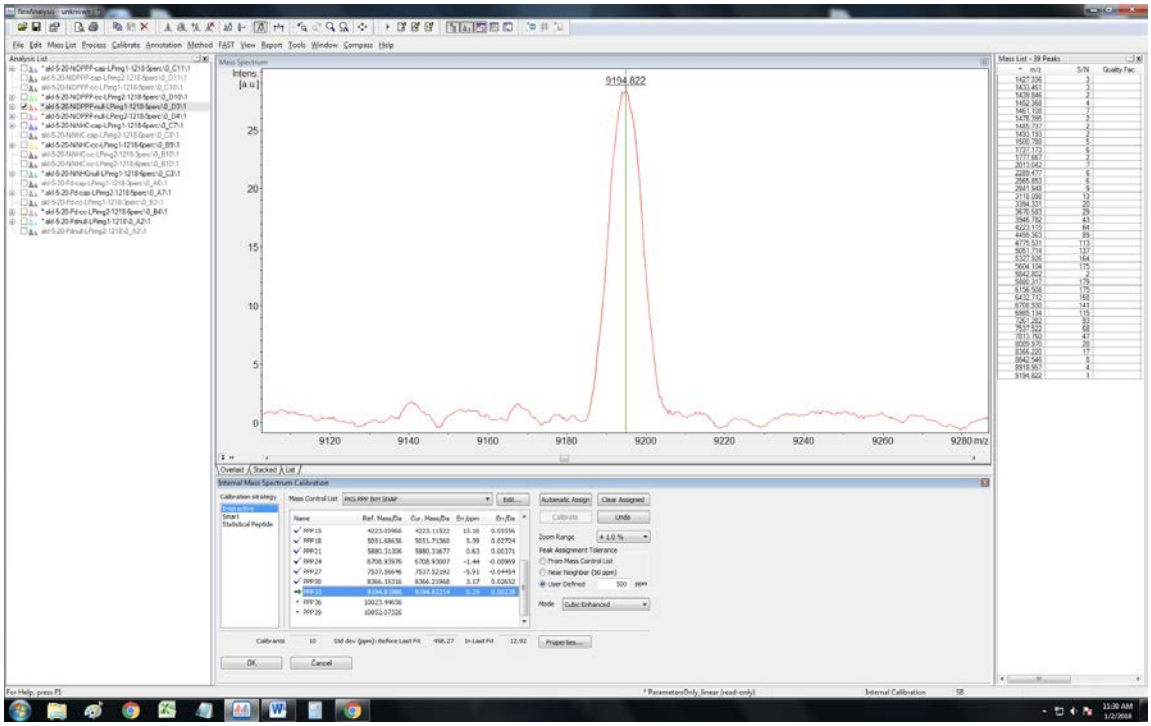


f. In the window, select the peak. The line will change from red to green, indicating you have calibrated the peak to where you clicked
    i. For SNAP - select the onset of the peak
    ii. Centroid - select the center of the peak

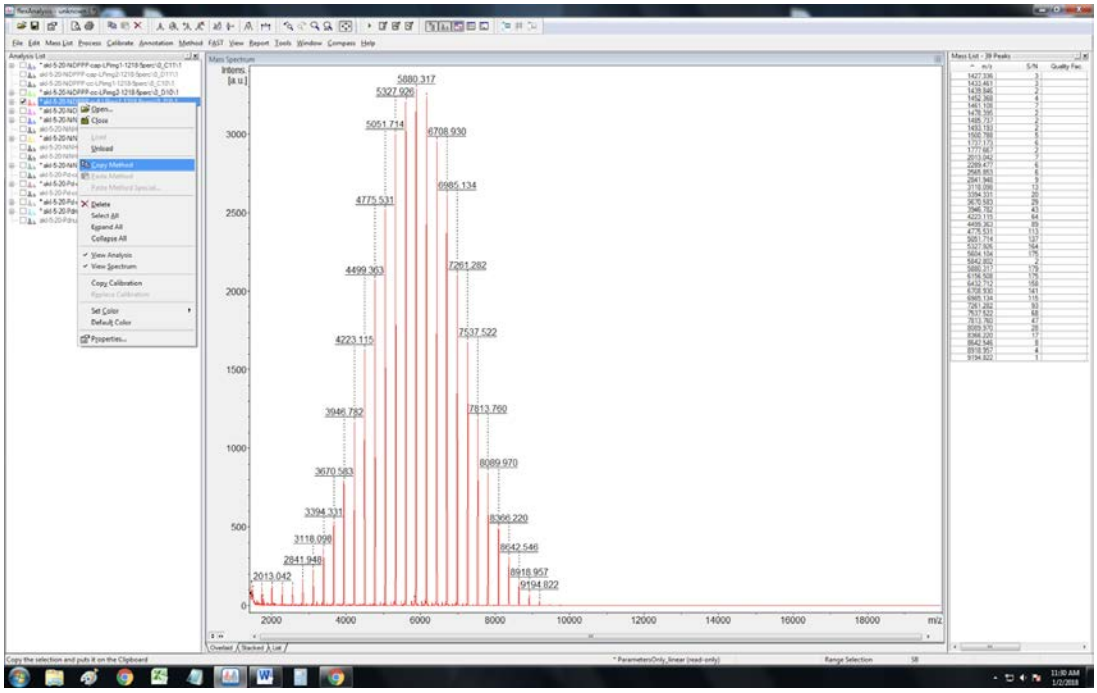


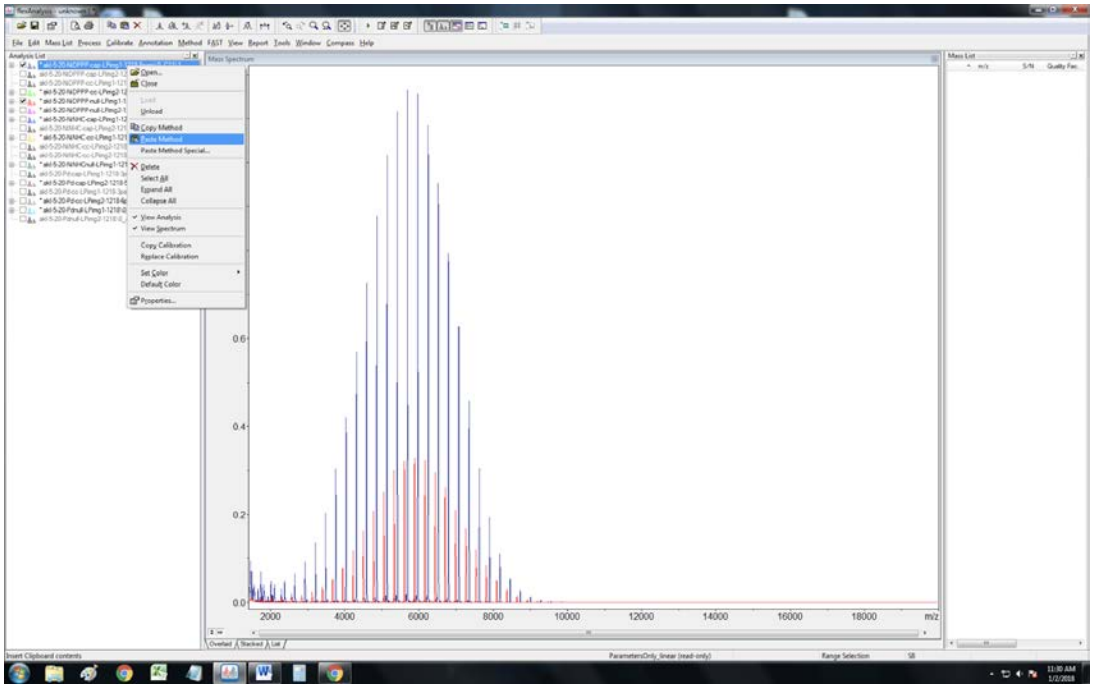
g. After selecting each peak click [calibrate] the green line should move to the center of your peak (see second img). Then click [OK]





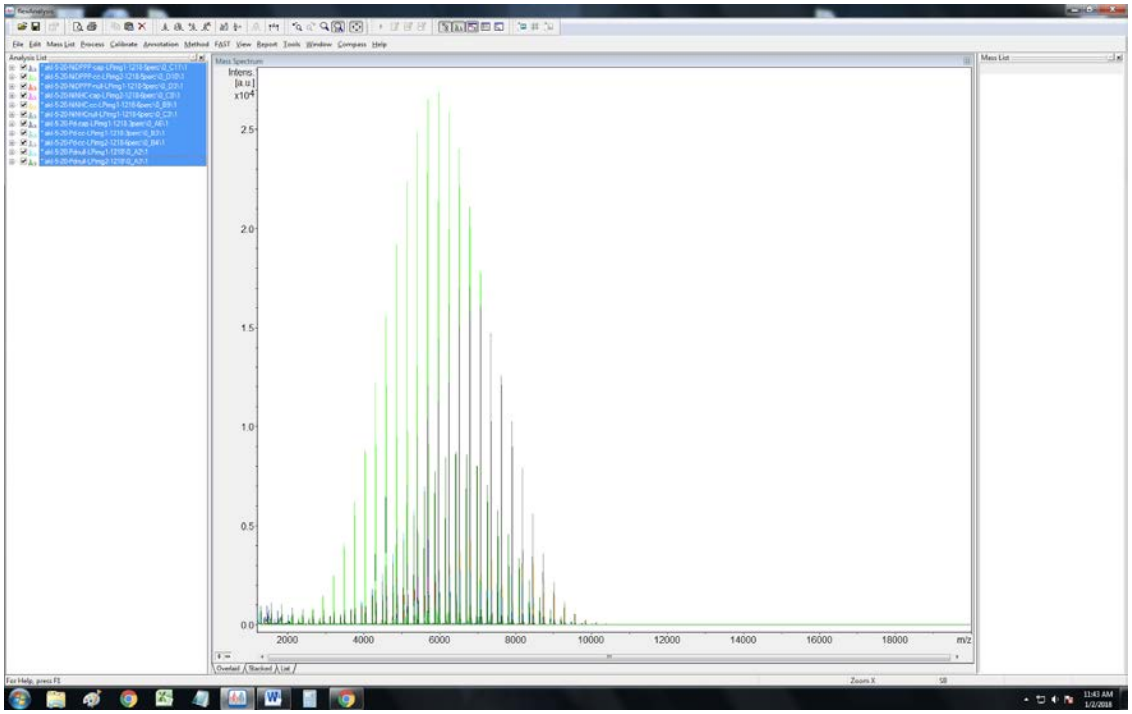
5. Highlight the data file, right click to Copy Method (peak finding method) & Copy Calibration - then Replace calibration and paste parameters on each sample (right click on their data file) - you should see the data shift slightly to match their calibrated curves.

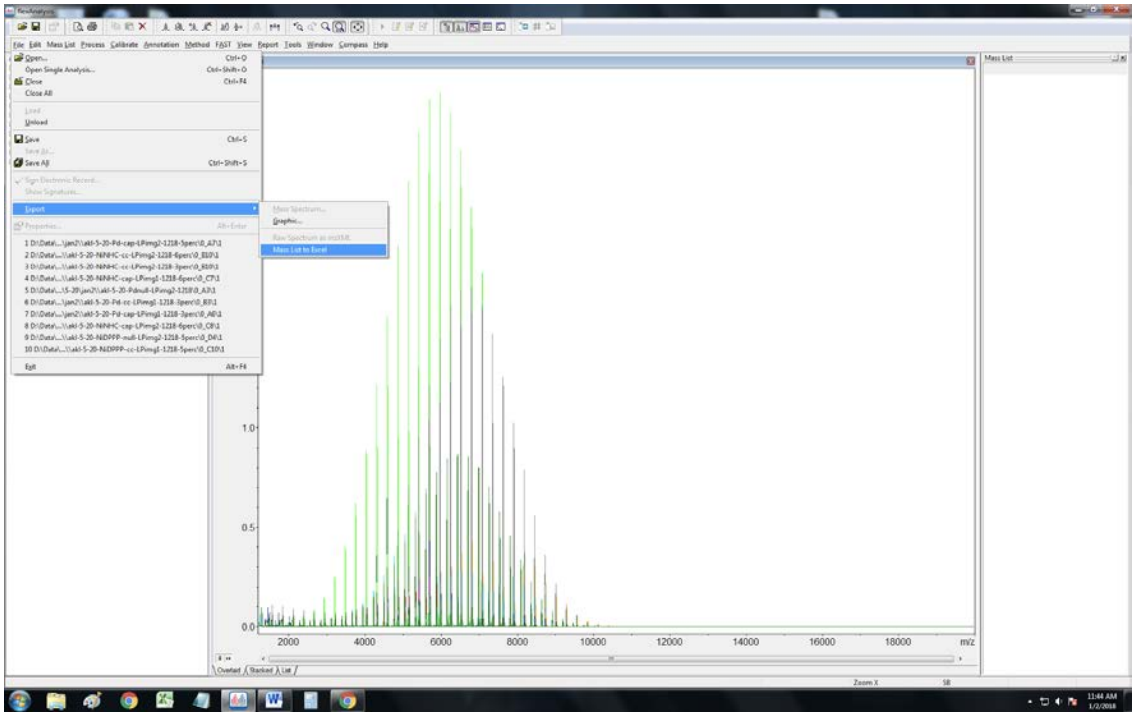




6. You have to Find Masses (F5) and process each plot
7. Export your data

a. File->export...
b. Masslist to excel: you can highlight all the samples and export their masslists to a single excel document (tab separated)



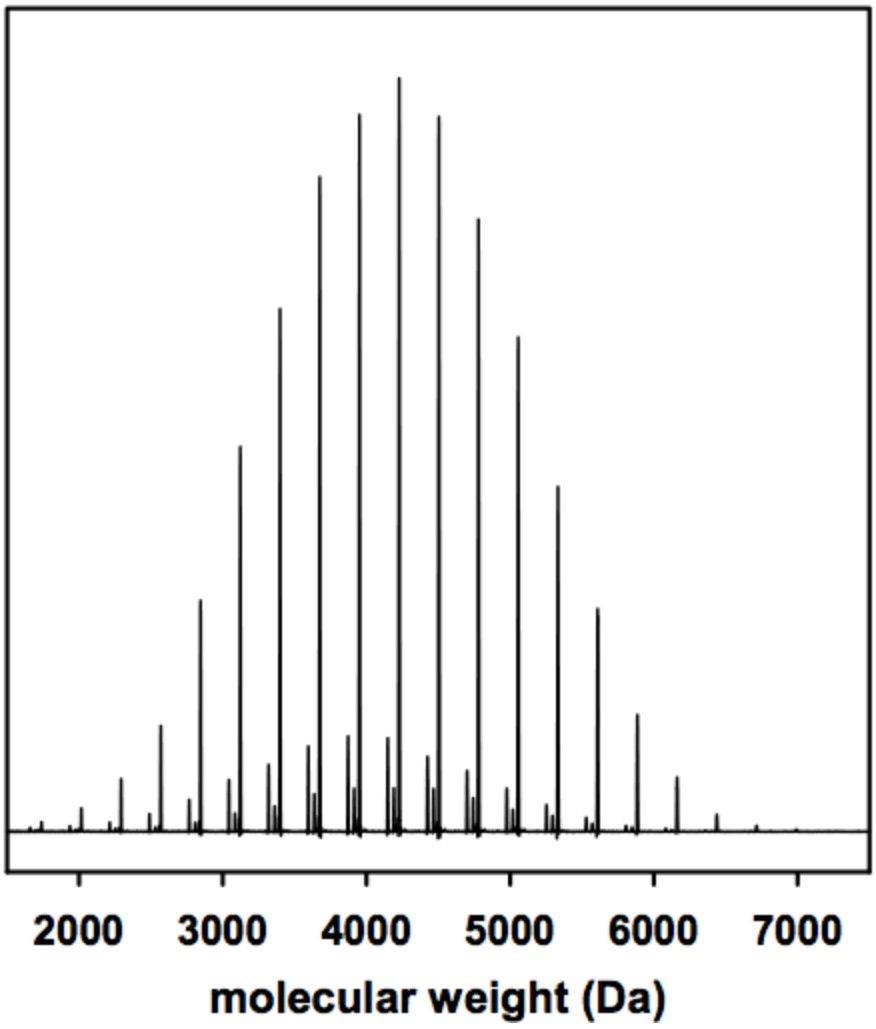


c. Mass Spectrum: Txt files of the spectra are saved separately and worked-up in sigmaplot (one at a time)

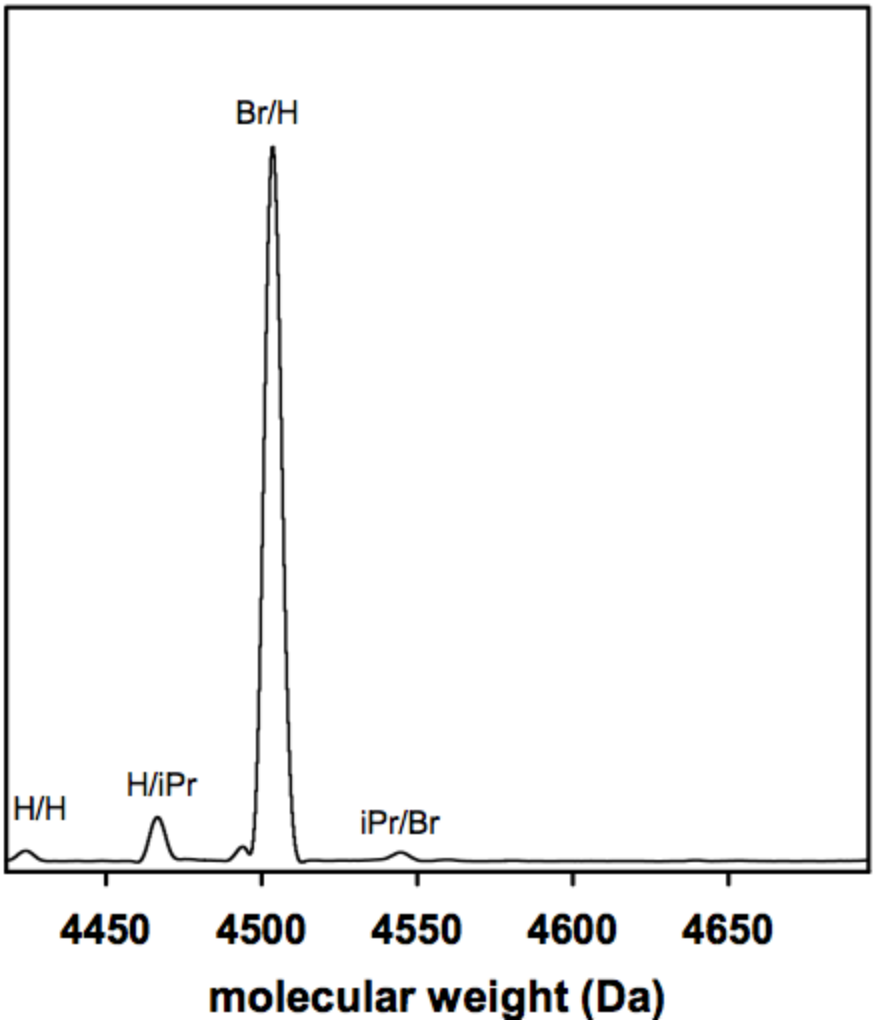
8. To make your life easier - load all files immediately into your electronic notebook

Data Work-up Part 2: sigmaplot

9. Open .txt files of your spectrum (cntrl L), your X and Y columns are *white space separated*
    a. To save plots as EPS (suggested), click Export. Make sure you *uncheck* the only export highlighted data only box
10. Plot using a Multiple Horizontal Step Plot.
    a. Show the full curve
        i. Make sure the full baseline and top of peak are visible



b. Zoom in to show the center of the peak and/or other points in the curve of interest typically a single repeat unit is a good X-axis window
    i. e.g., for poly(3-decyllthiophene) your window should be ~223 Da to encompass a full repeat unit. Note: if peaks are cut off (i.e., H/H or Br/Br) extend the window slightly to contain the full peak



c. Helpful labels
    i. Peaks (e.g., H/Br)
    ii. Degree of polymerization (DP)
    iii. Conditions of experiment of laser power (e.g., RP or LP)

11. Export the graphs and save them in an easy to find place and in your electronic notebook

Data Work-up Part 2: excel

Use excel to determine the relative percentages of your masses. It is important to analyze both the masslists *and* the spectrum, this way you can determine more clearly which peaks are *real* (i.e., not baseline noise or split peaks counted as two separate peaks). Tyler Lopez (McNeil lab member) made a helpful spreadsheet to analyze the sum of each repeat unit over the whole spectrum. When using this sheet, make sure to choose the right repeat unit values (recall SNAP vs Centroid).